Short- and Long-Term Immune Responses of CD-1 Outbred Mice to the Scrub Typhus DNA Vaccine Candidate: p47Kp

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ABSTRACT: *Orientia tsutsugamushi* is an obligate intracellular bacterium that is the causative agent of scrub typhus. To develop an effective vaccine to prevent or ameliorate scrub typhus, knowledge of the protective immune response to *O. tsutsugamushi* needs to be ascertained. Our laboratory has demonstrated that the DNA vaccine vector pVR1012 carrying the *O. tsutsugamushi* Karp strain 47-kDa protein gene (p47Kp) consistently provides outbred mice protection against homologous challenge.

KEYWORDS: CD-1 mice; immunization; p47Kp; scrub typhus; vaccine

INTRODUCTION

The host immune response to *Orientia tsutsugamushi*, an obligate intracellular bacterium that is the causative agent of scrub typhus, is believed to be mediated by both the humoral and cellular arms of the immune system, with greater importance associated with the latter.[1,2] To develop an effective vaccine to prevent or ameliorate scrub typhus, knowledge of the protective immune response to *O. tsutsugamushi* needs to be ascertained. Our laboratory has demonstrated that the DNA vaccine vector pVR1012 carrying the *O. tsutsugamushi* Karp strain 47-kDa protein gene (p47Kp) consistently provides outbred mice protection against homologous challenge (W-M. Ching *et al*., unpublished data). To assist in characterizing the immune response to this successful immunization strategy, we have evaluated the induction of antigen-specific antibody, and IFNγ, IL-4, and IL-13 secreting spleen cells derived from p47Kp-immunized mice at 4, 8, and 12 weeks and 6 months after immunization.

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METHODS

Seven- to eight-week-old female Swiss CD-1 outbred mice were inoculated intramuscularly with 100 μg of p47Kp once, twice, or three times at 4-week intervals before spleens were collected and processed. Spleen cells from immunized mice were evaluated for the presence of IFNγ, IL-4, and IL-13 secreting cells induced by *in vitro* stimulation with recombinant 47-kDa antigen from the Karp strain of *O. tsutsugamushi* (Kp r47) using cytokine-specific ELISpot assays. These immune responses were measured at 4 weeks and 6 months after the last immunization. The number of IFNγ, IL-4, and IL-13 producing mouse spleen cells were determined by ELISpot assays that utilized cytokine-specific monoclonal antibodies. Spleen cells (0.5 and 1.0×10^6 cells/well) were incubated with 1.0 μg/well of Kp r47 for 48 h. After washing, detection antibodies were added, the plates were incubated overnight, and then phosphatase-labeled streptavidin was added for 2 h. Following washing, the colored spots produced after adding BCIP/NBT phosphatase substrate were measured using the CLT ImmunoSpot Analyzer (Cellular Technology Ltd., Cleveland, OH). Sera from blood collected at 3 and 7 weeks and 6 months after immunization were assessed for antigen-specific IgG by ELISA similar to the procedure described previously,[2] except that 0.3 μg/well of Kp r47 antigen and an anti-mouse IgG antibody conjugated to HRP (KPL, Gaithersburg, MD) were used.

RESULTS

Mice immunized for 4, 8, or 12 weeks with a single dose of p47Kp induced a greater number of antigen-specific IFNγ producing cells than those from control mice immunized with vector alone (FIG. 1A). However, mice immunized for 8 weeks with two doses or 12 weeks with three doses of p47Kp produced a much greater number of antigen-specific IFNγ producing cells than the single immunizations (FIG. 1A). In addition to IFNγ producing spleen cells, IL-4 and IL-13 secreting cells were detected, especially from mice with two- and three-dose immunizations with p47Kp (FIG. 1A). Similar ELISpot results were obtained 6 months following the last immunization injection (FIG. 1B).

Antibody levels were either not detectable or very low at 3 weeks after immunization. However, antigen-specific antibody levels at 7 weeks ranged from a geometric mean titer of 635 for a single immunization to ≥6400 for three immunizations. Similar antibody levels were seen at 6 months (data not shown).

CONCLUSIONS

In addition to a strong antibody response, these results demonstrate that IFNγ and to a lesser extent IL-4 and IL-13 secreting spleen cells are induced *in vitro* in an antigen-specific manner following immunization of outbred CD-1 mice with the scrub typhus DNA vaccine candidate p47Kp. Moreover, CD-1 mice similarly immunized have been shown to be protected from homologous challenge (W-M. Ching *et al*., unpublished data). Last and more importantly, this study shows that the strong antigen-specific immune responses to p47Kp were still observable 6 months

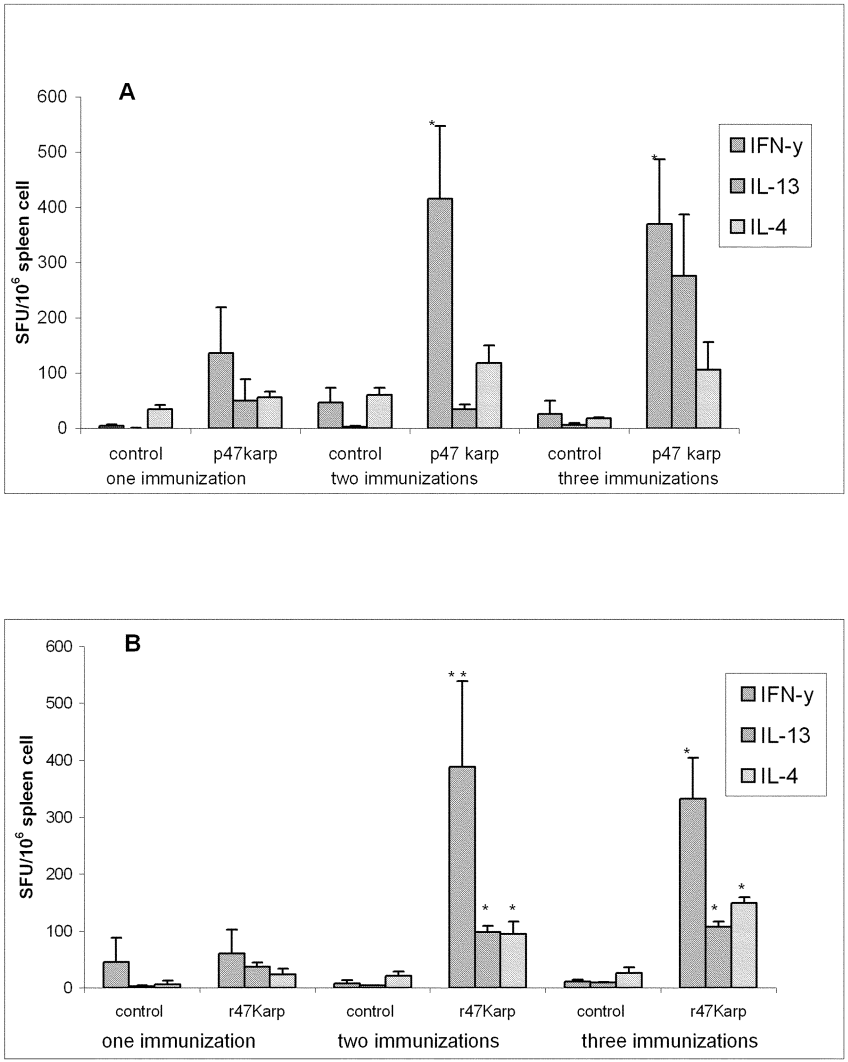

FIGURE 1. Antigen-specific spleen cell cytokine responses following multiple immunizations (1–3) at 4-week intervals. Spot forming units (SFU) are the number of cytokine producing cells reported for 1×10^6 spleen cells. Results are derived from spleen cells of mice that were immunized at **(A)** 4 weeks and **(B)** ~6 months earlier. Data shown represent the mean values plus SDs of 3 mice. Student's *t*-tests were used to calculate *P* values: $*P < 0.05$; $**P < 0.07$.

following immunization, suggesting that memory lymphocytes were generated by this vaccine candidate.

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DISCLAIMER

The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Department of Defense at large. The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals" (1996, Institute of Laboratory Animals Resources, National Research Council, National Academy Press, Washington, D.C.).

[*Competing interests statement:* The authors state that they have no competing interests.]

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